A TECHNIQUE FOR STAINING, DISSECTING, AND MOUNTING THE MALE GENITALIA OF BEETLES

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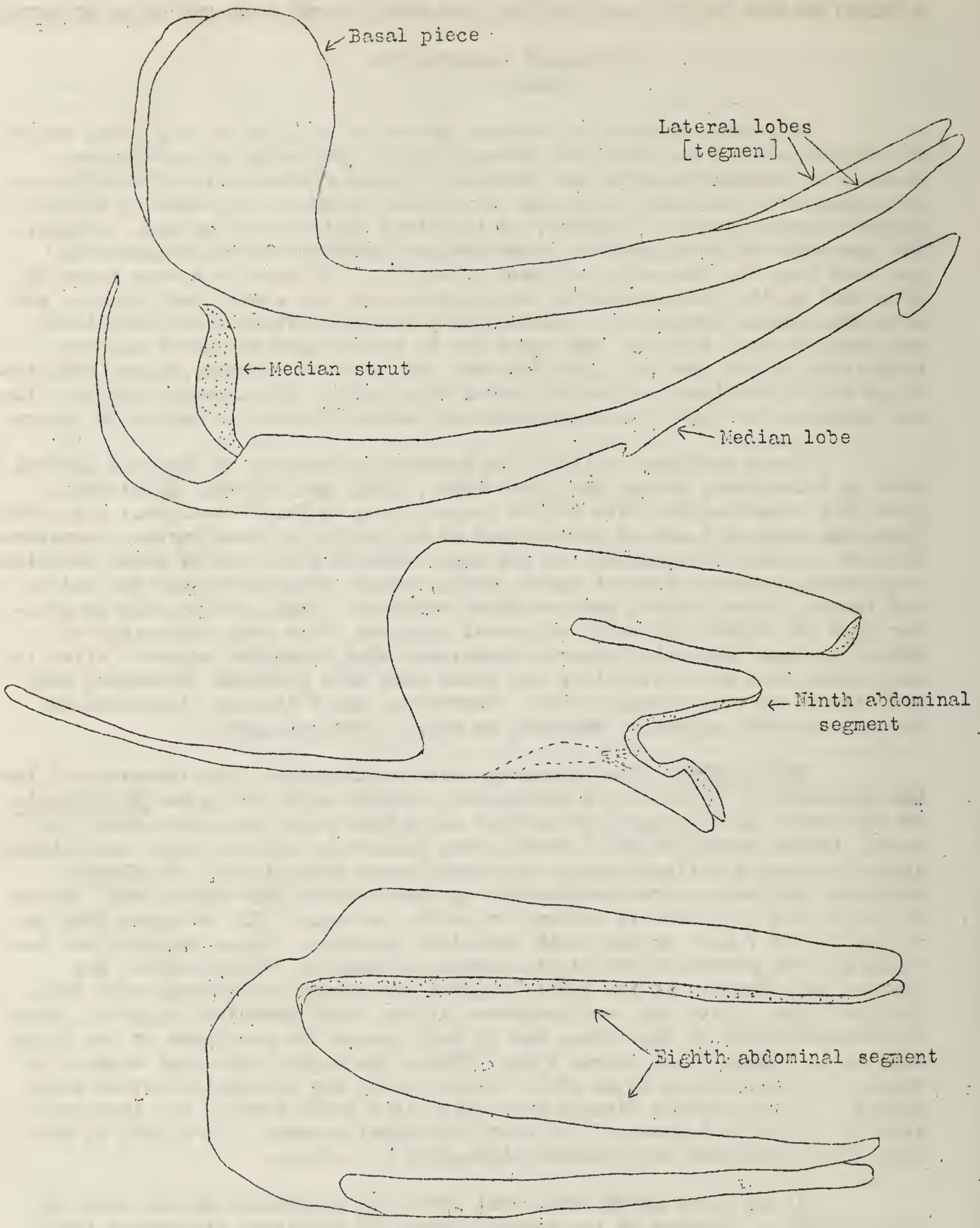
The classification of certain groups of beetles is to a large extent becoming dependent on characters offered by the genitalia, primarily the males, but more and more by the females. In some respects, this is unfortunate, because of the additional time which must be spent in preparing adequate mounts of these organs. However, it is a fact that in some groups, at least, the genitalia offer characters which are more positive for identification purposes than the characters offered externally. It must be always borne in mind that in the final analysis, these characters are really only another set of morphological structures. Perhaps they are less affected by nutritional and environmental factors, and hence can be relied upon to give a clearer definition of the species. But they are not immune to change, slight modifications and alterations, as are all other structures. So at best, they are, like all taxonomy, but man's interpretation of natures disorderly scheme of things.

Sharp and Muir in 1912 [The Comparative Anatomy of the male genital tube in Coleoptera, Trans. Ent. Soc. Lond., 1912, pp. 477-642, 37 plates], gave us a comprehensive view of the genital tube as found throughout the order. They also gave us a set of terms which we can apply to these various structures. However, due to the magnitude of the work, lack of time, and of vital practical importance, probably lack of space, these authors considered only the median and lateral lobes forming the so-called aedeagus. They mention only in passing that the eighth and ninth abdominal segments offer many characters of value. It has been this author's experience that these two segments offer in many cases much more diversity, and hence many more positive characters than are offered by the aedeagus alone. Therefore, the following discussion includes these two abdominal segments as well as the aedeagus.

MOROPHOLOGY: The following sketches illustrate the structure of the male genitalia as found in the Heteromera, specifically, the genus Rhinoplatia of the family OEdemeridae. It will be noted here that the median lobe fits into a trough formed by the lateral lobes [tegmen]. In this case, the lateral lobes are poorly differentiated, in other groups they are well developed processes projecting from the base along each side of the median lobe. These two parts are collectively refered to as the aedeagus. The aedeagus fits into the collar formed by the ninth abdominal segments. These together are between the two plates of the eighth abdominal segment. In this case, the tergite and sternite of the eighth segment are deeply emarginate, each forming two lobes. Note the long apodemere of the ninth abdominal segment. This is characteristic of the order, but in many groups the remainder of the ninth segment is reduced to a narrow ring. Often, the eighth abdominal segment is simply two flat plates which offer little in the way of identification characters. In the example figured here, this is a small lobe on the inner surface of the ventral plate of the ninth abdominal segment. This lobe is subject to considerable modification throughout the group.

It is these parts then, that should be considered in the study of the genitalia. Because of the several layers of chitinous structures involved, the author has found it an advantage in studying these parts to dissect the membranes holding them together and separate the individual segments. In some cases, however, this is not necessary as the entire set of structures may be stretched out into a long series, with the membrane holding them to-



LATERAL VIEW OF MALE GENITALIA
(Dorsal surface towards top of drawing)

gether, without overlapping of the parts.

It must be mentioned here, that even by including the eighth and ninth abdominal segments in the study of the genitalia, the possibilities of finding valuable characters are not exhausted. If the membranes connecting these harder parts, the ducts leading into the parts, and the muscles connected to these parts were throughly studied, much more could be learned from these structures about affinities, etc., perhaps facts of a much more fundamental nature than any known at the moment.

TECHNIQUE: The first step in the preparation of the material for study is relaxing the specimen. This is done simply by putting the specimen in a beaker of hot, but not boiling water, after first removing the labels. Only one specimen at a time however, because of the danger of confusing the data, should be relaxed. The author has found it more convenient to relax specimens in this way, rather than placing a number of specimens in a relaxing jar over night, for two reasons. The first is, that there is always the danger of the labels becoming water soaked in a relaxing jar, and secondly, it is not always possible to determine the sex of the specimen before hand, and hence, the relaxing jar may be filled with females instead of males. By this faster method, a great number of specimens may be relaxed in a few minutes, and if they prove to be females, there has been little time lost.

After the specimen has been relaxed, the genitalia are removed by holding the specimens between the thumb and forefinger under the low power of the binocular microscope and extracting the genitalia by means of a finely ground insect pin. Care must be taken not to cut or pierce the structures with the pin. If the specimen is throughly relaxed, the genitalia will easily pull out to a point where it may be cut off with fine scissors or pulled off with a pair of forceps.

After the genitalia are removed they should be placed in a small watch glass of pure water. The author has found that the two cm. size watch glasses used by the U.S. Department of Agriculture, Bureau of Plant Quarantine, are the most economical to use. A number is given to both the specimen and to the removed genitalia, and this number recorded in a record book. If the plates of the seventh abdominal segment have been removed with the genitalia, as is often the case, these may be removed while in the water, and the structure teased apart somewhat before it is placed in KOH. This will facilitate the penetration of the KOH and hasten the eating away of the muscles and membrane. A ten percent solution of potassium hydroxide is used to dissolve out the muscle and membrane. This is heated in a small casserole with a cover. The exact concentration of the KOH is relatively unimportant, unless the structure is particularly small and delicate. The continued heating of the KOH soon boils away a great deal of water, thus greatly increasing the concentration of the KOH, making it necessary to frequently add water. By using a covered casserole, this is somewhat overcome. But the change in concentration has no bad effects on the genitalia if a close watch is kept. Here again time is saved. If the genitalia were soaked in cold KOH over night as is usually recommended, much more time is necessary to prepare a series of slides, and also, what is more important, the progress of dissolving the undesired tissues cannot be as closely checked.

It is often desirable in the case of small or weakly chitinized genitalia to stain with Gage's stain to bring out the structure. Gage's stain is prepareed as follows:

Acid fuchsin . . . . . 0.5 gram
10 percent hydrochloric acid . . . . . 25.0 cc.
(Add 10 cc. of concentrated HCl [Sp. gr. 1.18] to 90 cc. of distilled water.)
Distilled water . . . . . 300.0 cc.

This stock solution is diluted, one drop of stain to five drops of water.

After the genitalia are removed from the KOH, the length of time being dependent on the amount of muscle and membrane necessary to remove, it is placed in acetic alcohol (3 parts of 50 percent ethyl alcohol, 1 part acetic acid). This has the additional advantage of expanding and extending the organ as well as neutralizing the KOH and stopping the action. It is also necessary because Gage's stain is colorless in a base solution. From the acetic alcohol, it is placed directly into the stain. The stain is diluted, and the watch glass covered and left for 12 hours.

Dissection may be done either before or after the staining, which ever is more convenient. Dissection should be done only where it is necessary to see the parts clearly, and if done, care must be taken to observe the relationship of the parts. It is well to sketch the parts as observed during the dissection. Sometimesit is an advantage to leave the dissecting until after the structures have been cleared in xylene.

After removing from the stain, place in water to remove the excess stain, and then place in 95% alcohol, except in the case of very delicate specimens in which case placing directly in a concentration of alcohol may cause some distortion. From 95% alcohol, place in carbo-xylene, which is prepared from a solution of carbolic acid, one part and xylene, one part. From the carbo-xylene, place in pure xylene, and then mount in balsam. The length of time necessary to leave the specimen in each solution must be determined by experience, but in general, five minutes in each is sufficient. Often, if there is little to prevent a free penetration of the solution, much less time is necessary for each step.

It is always well, before making a permanent slide of a specimen, to make a sketch of the structure while it can still be freely moved. Often, if this is not done, important structures, or a least form and relationship is overlooked.

As with any attempt to outline technique, this is little more than a sketch of some of the points to be considered. Each technician must work out the details for himself. Each group presents certain problems peculiar to that group, and must be overcome by a modification of technique. Therefore it is important to be always alert, and ready to make changes to suit the case. Do not let the technique become the end, but rather carefully prepared material which will serve to best advantage the worker in carrying on his research should be the end. Remember that an extruded, dried genitalia still attached to the specimen may serve the purpose just as well as an elaborately prepared and stained slide. It depends solely upon the complexity of the structure and the fineness of the characters necessary to employ in separating the species.